

SEQUENCE LISTING

<110> Duke University
 Chuan-Yuan, Li
 5 Xiuwu, Zhang
 <120> GENERATION OF RECOMBINANT ADENO-ASSOCIATED VIRAL VECTORS BY A
 COMPLETE ADENOVIRUS-MEDIATED APPROACH
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 <150> US 60/349,532
 <151> 2002-01-18
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 Glu His Leu Pro Gly Ile Ser Asp Ser Phe Val Asn Trp Val Ala Glu
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 40 aag gaa tgg gag ttg ccg cca gat tct gac atg gat ctg aat ctg att 144
 Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile
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 45 gag cag gca ccc ctg acc gtg gcc gag aag ctg cag cgc gac ttt ctg 192
 Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu
 50 55 60
 50 acg gaa tgg cgc cgt gtg agt aag gcc ccg gag gcc ctt ttc ttt gtg 240
 Thr Glu Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val
 65 70 75 80
 55 caa ttt gag aag gga gag agc tac ttc cac atg cac gtg ctc gtg gaa 288
 Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Met His Val Leu Val Glu
 85 90 95
 60 acc acc ggg gtg aaa tcc atg gtt ttg gga cgt ttc ctg agt cag att 336
 Thr Thr Gly Val Lys Ser Met Val Leu Gly Arg Phe Leu Ser Gln Ile
 100 105 110
 cgc gaa aaa ctg att cag aga att tac cgc ggg atc gag ccg act ttg 384
 Arg Glu Lys Leu Ile Gln Arg Ile Tyr Arg Gly Ile Glu Pro Thr Leu
 115 120 125
 cca aac tgg ttc gcg gtc aca aag acc aga aat ggc gcc gga ggc ggg 432
 Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
 130 135 140

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	Ser Ala Cys Leu Asn Leu Thr Glu Arg Lys Arg Leu Val Ala Gln His	
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15	ctg acg cac gtg tcg cag acg cag gag cag aac aaa gag aat cag aat	624
	Leu Thr His Val Ser Gln Thr Gln Glu Gln Asn Lys Glu Asn Gln Asn	
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20	ccc aat tct gat gcg ccg gtg atc aga tca aaa act tca gcc agg tac	672
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25	atg gag ctg gtc ggg tgg ctc gtg gac aag ggg att acc tcg gag aag	720
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30	cag tgg atc cag gag gac cag gcc tca tac atc tcc ttc aat gcg gcc	768
	Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala	
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35	tcc aac tcg ccg tcc caa atc aag gct gcc ttg gac aat gcg gga aag	816
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40	att atg agc ctg act aaa acc gcc ccc gac tac ctg gtg ggc cag cag	864
	Ile Met Ser Leu Thr Lys Thr Ala Pro Asp Tyr Leu Val Gly Gln Gln	
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	Pro Val Glu Asp Ile Ser Ser Asn Arg Ile Tyr Lys Ile Leu Glu Leu	
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	Thr Lys Lys Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala	
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	Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Thr Val Pro	
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65	ttc tac ggg tgc gta aac tgg acc aat gag aac ttt ccc ttc aac gac	1104
	Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp	
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70	tgt gtc gac aag atg gtg atc tgg tgg gag gag ggg aag atg acc gcc	1152
	Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala	
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	Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg	
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	Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val	
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5	atc gtc acc tcc aac acc aac atg tgc gcc gtg att gac ggg aac tca	1296
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10	acg acc ttc gaa cac cag cag ccg ttg caa gac cgg atg ttc aaa ttt	1344
	Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe	
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15	gaa ctc acc cgc cgt ctg gat cat gac ttt ggg aag gtc acc aag cag	1392
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	Glu His Glu Phe Tyr Val Lys Lys Gly Gly Ala Lys Lys Arg Pro Ala	
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25	ccc agt gac gca gat ata agt gag ccc aaa cgg gtg cgc gag tca gtt	1536
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55	Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile	
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60	Glu Gln Ala Pro Leu Thr Val Ala Glu Lys Leu Gln Arg Asp Phe Leu	
	50 55 60	
	Thr Glu Trp Arg Arg Val Ser Lys Ala Pro Glu Ala Leu Phe Phe Val	
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10	Arg	Glu	Lys	Leu	Ile	Gln	Arg	Ile	Tyr	Arg	Gly	Ile	Glu	Pro	Thr	Leu
			115					120					125			
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		130					135					140				
20	Asn	Lys	Val	Val	Asp	Glu	Cys	Tyr	Ile	Pro	Asn	Tyr	Leu	Leu	Pro	Lys
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25	Thr	Gln	Pro	Glu	Leu	Gln	Trp	Ala	Trp	Thr	Asn	Met	Glu	Gln	Tyr	Leu
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30	Ser	Ala	Cys	Leu	Asn	Leu	Thr	Glu	Arg	Lys	Arg	Leu	Val	Ala	Gln	His
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40	Pro	Asn	Ser	Asp	Ala	Pro	Val	Ile	Arg	Ser	Lys	Thr	Ser	Ala	Arg	Tyr
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50	Gln	Trp	Ile	Gln	Glu	Asp	Gln	Ala	Ser	Tyr	Ile	Ser	Phe	Asn	Ala	Ala
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55	Ser	Asn	Ser	Arg	Ser	Gln	Ile	Lys	Ala	Ala	Leu	Asp	Asn	Ala	Gly	Lys
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60	Ile	Met	Ser	Leu	Thr	Lys	Thr	Ala	Pro	Asp	Tyr	Leu	Val	Gly	Gln	Gln
			275					280					285			
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 10 Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
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 15 Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
 385 390 395 400
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 420 425 430
 25 Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
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	Lys Glu Trp Glu Leu Pro Pro Asp Ser Asp Met Asp Leu Asn Leu Ile	
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	Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly	
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	485 490 495	
75	ccc agt gac gca gat ata agt gag ccc aaa cgg gtg cgc gag tca gtt	1536
	Pro Ser Asp Ala Asp Ile Ser Glu Pro Lys Arg Val Arg Glu Ser Val	
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15 ttc act cac gga cag aaa gac tgt tta gag tgc ttt ccc gtg tca gaa 1728
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Ser Gln Pro Val Ser Val Val Lys Lys Ala Tyr Gln Lys Leu Cys Tyr
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Gln Phe Glu Lys Gly Glu Ser Tyr Phe His Met His Val Leu Val Glu
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5 Pro Asn Trp Phe Ala Val Thr Lys Thr Arg Asn Gly Ala Gly Gly Gly
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10 Asn Lys Val Val Asp Glu Cys Tyr Ile Pro Asn Tyr Leu Leu Pro Lys
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15 Thr Gln Pro Glu Leu Gln Trp Ala Trp Thr Asn Met Glu Gln Tyr Leu
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Ser Ala Cys Leu Asn Leu Thr Glu Arg Lys Arg Leu Val Ala Gln His
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25 Pro Asn Ser Asp Ala Pro Val Ile Arg Ser Lys Thr Ser Ala Arg Tyr
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55 Thr Lys Lys Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
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Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Thr Val Pro
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15 Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
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30 Glu His Glu Phe Tyr Val Lys Lys Gly Gly Ala Lys Lys Arg Pro Ala
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35 Pro Ser Asp Ala Asp Ile Ser Glu Pro Lys Arg Val Arg Glu Ser Val
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55 Ser Gln Pro Val Ser Val Val Lys Lys Ala Tyr Gln Lys Leu Cys Tyr
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 10 Gln Trp Ile Gln Glu Asp Gln Ala Ser Tyr Ile Ser Phe Asn Ala Ala
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 15 Ser Asn Ser Arg Ser Gln Ile Lys Ala Ala Leu Asp Asn Ala Gly Lys
 35 40 45
 20 Ile Met Ser Leu Thr Lys Thr Ala Pro Asp Tyr Leu Val Gly Gln Gln
 50 55 60
 25 Pro Val Glu Asp Ile Ser Ser Asn Arg Ile Tyr Lys Ile Leu Glu Leu
 65 70 75 80
 Asn Gly Tyr Asp Pro Gln Tyr Ala Ala Ser Val Phe Leu Gly Trp Ala
 85 90 95
 30 Thr Lys Lys Phe Gly Lys Arg Asn Thr Ile Trp Leu Phe Gly Pro Ala
 100 105 110
 35 Thr Thr Gly Lys Thr Asn Ile Ala Glu Ala Ile Ala His Thr Val Pro
 115 120 125
 40 Phe Tyr Gly Cys Val Asn Trp Thr Asn Glu Asn Phe Pro Phe Asn Asp
 130 135 140
 45 Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
 145 150 155 160
 Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
 165 170 175
 50 Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
 180 185 190
 55 Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
 195 200 205
 60 Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
 210 215 220
 Glu Leu Thr Arg Arg Leu Asp His Asp Phe Gly Lys Val Thr Lys Gln
 225 230 235 240

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Cys Val Asp Lys Met Val Ile Trp Trp Glu Glu Gly Lys Met Thr Ala
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 5 Lys Val Val Glu Ser Ala Lys Ala Ile Leu Gly Gly Ser Lys Val Arg
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 10 Val Asp Gln Lys Cys Lys Ser Ser Ala Gln Ile Asp Pro Thr Pro Val
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 15 Ile Val Thr Ser Asn Thr Asn Met Cys Ala Val Ile Asp Gly Asn Ser
 195 200 205
 20 Thr Thr Phe Glu His Gln Gln Pro Leu Gln Asp Arg Met Phe Lys Phe
 210 215 220
 Glu Leu Thr Arg Arg Leu Asp His Asp Phe Gly Lys Val Thr Lys Gln
 225 230 235 240
 25 Glu Val Lys Asp Phe Phe Arg Trp Ala Lys Asp His Val Val Glu Val
 245 250 255
 30 Glu His Glu Phe Tyr Val Lys Lys Gly Gly Ala Lys Lys Arg Pro Ala
 260 265 270
 35 Pro Ser Asp Ala Asp Ile Ser Glu Pro Lys Arg Val Arg Glu Ser Val
 275 280 285
 40 Ala Gln Pro Ser Thr Ser Asp Ala Glu Ala Ser Ile Asn Tyr Ala Asp
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 Arg Tyr Gln Asn Lys Cys Ser Arg His Val Gly Met Asn Leu Met Leu
 305 310 315 320
 45 Phe Pro Cys Arg Gln Cys Glu Arg Met Asn Gln Asn Ser Asn Ile Cys
 325 330 335
 50 Phe Thr His Gly Gln Lys Asp Cys Leu Glu Cys Phe Pro Val Ser Glu
 340 345 350
 55 Ser Gln Pro Val Ser Val Val Lys Lys Ala Tyr Gln Lys Leu Cys Tyr
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 60 Ile His His Ile Met Gly Lys Val Pro Asp Ala Cys Thr Ala Cys Asp
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 5 <213> adeno-associated virus 2

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 30 <211> 735
 <212> PRT
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 45 Lys Pro Ala Glu Arg His Lys Asp Asp Ser Arg Gly Leu Val Leu Pro
 35 40 45

 50 Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
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 55 Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
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 60 Arg Gln Leu Asp Ser Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
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 Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
 100 105 110

 Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro

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15	Lys Ala Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln Thr 165 170 175		
20	Gly Asp Ala Asp Ser Val Pro Asp Pro Gln Pro Leu Gly Gln Pro Pro 180 185 190		
25	Ala Ala Pro Ser Gly Leu Gly Thr Asn Thr Met Ala Thr Gly Ser Gly 195 200 205		
30	Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ser 210 215 220		
35	Ser Gly Asn Trp His Cys Asp Ser Thr Trp Met Gly Asp Arg Val Ile 225 230 235 240		
40	Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu 245 250 255		
45	Tyr Lys Gln Ile Ser Ser Gln Ser Gly Ala Ser Asn Asp Asn His Tyr 260 265 270		
50	Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe His 275 280 285		
55	Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn Trp 290 295 300		
60	Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln Val 305 310 315 320		
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	Thr Ser Thr Val Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu Pro Tyr 340 345 350		
	Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala Asp 355 360 365		
	Val Phe Met Val Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly Ser		

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10	Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe Glu 405 410 415		
15	Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp Arg 420 425 430		
20	Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser Arg Thr 435 440 445		
25	Asn Thr Pro Ser Gly Thr Thr Thr Gln Ser Arg Leu Gln Phe Ser Gln 450 455 460		
30	Ala Gly Ala Ser Asp Ile Arg Asp Gln Ser Arg Asn Trp Leu Pro Gly 465 470 475 480		
35	Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Ser Ala Asp Asn Asn 485 490 495		
40	Asn Ser Glu Tyr Ser Trp Thr Gly Ala Thr Lys Tyr His Leu Asn Gly 500 505 510		
45	Arg Asp Ser Leu Val Asn Pro Gly Pro Ala Met Ala Ser His Lys Asp 515 520 525		
50	Asp Glu Glu Lys Phe Phe Pro Gln Ser Gly Val Leu Ile Phe Gly Lys 530 535 540		
55	Gln Gly Ser Glu Lys Thr Asn Val Asp Ile Glu Lys Val Met Ile Thr 545 550 555 560		
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65	Gly Ser Val Ser Thr Asn Leu Gln Arg Gly Asn Arg Gln Ala Ala Thr 580 585 590		
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75	Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His Thr 610 615 620		
80	Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu Lys		

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10	Pro	Ser	Thr	Thr	Phe	Ser	Ala	Ala	Lys	Phe	Ala	Ser	Phe	Ile	Thr	Gln
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15	Tyr	Ser	Thr	Gly	Gln	Val	Ser	Val	Glu	Ile	Glu	Trp	Glu	Leu	Gln	Lys
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	<211>	598														
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60	Ala	Asp	Gly	Val	Gly	Asn	Ser	Ser	Gly	Asn	Trp	His	Cys	Asp	Ser	Thr
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65	Trp	Met	Gly	Asp	Arg	Val	Ile	Thr	Thr	Ser	Thr	Arg	Thr	Trp	Ala	Leu
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 10 Met Gly Asp Arg Val Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro
 35 40 45
 15 Thr Tyr Asn Asn His Leu Tyr Lys Gln Ile Ser Ser Gln Ser Gly Ala
 50 55 60
 20 Ser Asn Asp Asn His Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe
 65 70 75 80
 Asp Phe Asn Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg
 85 90 95
 25 Leu Ile Asn Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys
 100 105 110
 30 Leu Phe Asn Ile Gln Val Lys Glu Val Thr Gln Asn Asp Gly Thr Thr
 115 120 125
 35 Thr Ile Ala Asn Asn Leu Thr Ser Thr Val Gln Val Phe Thr Asp Ser
 130 135 140
 40 Glu Tyr Gln Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu
 145 150 155 160
 Pro Pro Phe Pro Ala Asp Val Phe Met Val Pro Gln Tyr Gly Tyr Leu
 165 170 175
 45 Thr Leu Asn Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys
 180 185 190
 50 Leu Glu Tyr Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Thr
 195 200 205
 55 Phe Ser Tyr Thr Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His
 210 215 220
 Ser Gln Ser Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu
 225 230 235 240
 60 Tyr Tyr Leu Ser Arg Thr Asn Thr Pro Ser Gly Thr Thr Thr Gln Ser
 245 250 255

Arg Leu Gln Phe Ser Gln Ala Gly Ala Ser Asp Ile Arg Asp Gln Ser
 260 265 270
 5 Arg Asn Trp Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys
 275 280 285
 10 Thr Ser Ala Asp Asn Asn Asn Ser Glu Tyr Ser Trp Thr Gly Ala Thr
 290 295 300
 15 Lys Tyr His Leu Asn Gly Arg Asp Ser Leu Val Asn Pro Gly Pro Ala
 305 310 315 320
 20 Met Ala Ser His Lys Asp Asp Glu Glu Lys Phe Phe Pro Gln Ser Gly
 325 330 335
 Val Leu Ile Phe Gly Lys Gln Gly Ser Glu Lys Thr Asn Val Asp Ile
 340 345 350
 25 Glu Lys Val Met Ile Thr Asp Glu Glu Glu Ile Arg Thr Thr Asn Pro
 355 360 365
 30 Val Ala Thr Glu Gln Tyr Gly Ser Val Ser Thr Asn Leu Gln Arg Gly
 370 375 380
 35 Asn Arg Gln Ala Ala Thr Ala Asp Val Asn Thr Gln Gly Val Leu Pro
 385 390 395 400
 40 Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp
 405 410 415
 Ala Lys Ile Pro His Thr Asp Gly His Phe His Pro Ser Pro Leu Met
 420 425 430
 45 Gly Gly Phe Gly Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn
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 465 470 475 480
 Glu Trp Glu Leu Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile
 485 490 495
 60 Gln Tyr Thr Ser Asn Tyr Asn Lys Ser Val Asn Val Asp Phe Thr Val
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5 Leu Thr Arg Asn Leu
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 20 gccaactcca tcactagggg ttctt 145

25 <210> 13
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 <212> DNA
 <213> adeno-associated virus 2

30 <400> 13
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 agcgcgaga gagggagtgg ccaa 144

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 <211> 31
 <212> DNA
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 45 <223> corresponds to base pairs 318-339 of AAV with an EcoR V site at
 the 5' end

50 <400> 14
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55 <210> 15
 <211> 35
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 <213> adeno-associated virus

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 <223> corresponds to base pairs 1812 to 1846 of AAV

<220>
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<222> (20)..(20)
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 <221> mutation
 5 <222> (23)..(23)
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 <211> 35
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 25
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 55
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 <223> corresponds to base pairs 2261-2289 of AAV
 60
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 <222> (28)..(53)
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10  <220>
    <221> misc_feature
    <222> (1)..(28)
    <223> corresponds to base pairs 4446-4467 of AAV with a BamHI site at
15  the 3' end

    <400> 19
    ggatcccgca gagaccaaag ttcaactg      28

20  <210> 20
    <211> 27
    <212> DNA
    <213> adeno-associated virus

25  <220>
    <221> misc_feature
    <222> (1)..(27)
30  <223> corresponds to base pairs 1852 to 1878, BamHI site at the 5' end

    <400> 20
    agtcagttgc gcagccatcg acgtcag      27

35  <210> 21
    <211> 35
    <212> DNA
    <213> adeno-associated virus

40  <220>
    <221> misc_feature
    <222> (1)..(35)
45  <223> corresponds to base pairs 4302 to 4329, with a Not I site at the
    3' end

    <400> 21
    gcggccgctt aacagacttg ttgtagttgg aagtg      35

50  <210> 22
    <211> 592
    <212> DNA
55  <213> Homo sapiens

    <400> 22
    gcgtgaagag ctgcagtgtc actcttaaag ctgaattaat ctctgccatt ccttaaggaa      60

60  acaggcaact gtcttaaaac cgtgggttttg aaaatatttt gttcaagata aaactgtttt      120
    aagatatatg tatatatatc ttatatatct gtattcgcat ggtaacatat cttcgggtctt      180
    cctgccgctg ggctctcagc ggccctccaa ggcagcccgcc aggcccgctgc tcgcctcagg      240

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	gatcctccac agccccgggg agaccttgcc tctaaagttg ctgcttttgc agctctgcca	300
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